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User's Guide ▶▶▶

Global Genomics Partner

AccuPrep®
96 Genomic DNA Extraction Kit

Cat. No.: K-3032-2

BIONEER
bioneer corporation

V109C3

Safety Warnings and Precautions
This kit is for research use only, and should not be used for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Always wear gloves when treating irritants or harmful reagents.

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All aspects of our quality management system, from product development and production to quality assurance and supplier qualification, have been certified to meet world-class standards.

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Each lot of BIONEER's product is tested in our quality control team as raw material prior to purchase. Acceptable lots are processed and tested again as finished product.
Prior to purchase, each lot of the product is tested by BIONEER's quality control team as raw materials. The acceptable lots are processed and retested as a finished product.

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AccuPrep® 96 Genomic DNA Extraction Kit

Technical Manual

I. Introduction

AccuPrep® 96 Genomic DNA Extraction Kit allows fast and easy preparation of genomic DNA from 96 different samples, such as whole blood, lymphocyte and cell media.
It has high concentrated chaotropic salt bound to fixed glass fiber in column, and protein and other contaminants are eliminated through washing step. DNA is isolated and purified by elution step. The kit is safe and convenient because organic solvent or ethanol precipitation is not needed in all steps. Samples possibly to be applied for DNA extraction by the kit are citrate, whole blood, buffy coat, lymphocytes, plasma, serum, body fluids or cultured cell which was treated with EDTA.

Advantages :

1.Easy and fast extraction of Genomic DNA from different sources

2. Removal of protein and other contaminants that inhibit PCR reaction increases product yield.

3.Damage and low yield of DNA are minimized because the all steps proceed without organic solvent and ethanol precipitation process.

4.Extracted Genomic DNA can be used for various experiments.

II. Kit Components

192 samples are included and the quality is guaranteed for 1 year from manufacturing date.

K-3032-2 *AccuPrep*® 96 Genomic DNA Extraction Kit

Kit Components	
Proteinase K, lyophilized	25 mg X 4
Freeze-dried Proteinase K 25mg is contained in a tube. Storage at -20℃ is recommended to keep its activity after it is completely dissolved in 1.25ml of sterile water. Dissolved Proteinase K should be kept separately in tubes in order to protect its activity. Repeated freezing and melting can lower the activity level.	
Binding buffer (**B**)	50 ml
Use after shaking. Storage at room temperature is recommended. *Caution : Do not mix Proteinase K with Binding buffer directly.	
Washing buffer 1 (**W1**)	80 ml
W1 buffer is supplied as a concentrate. At first use, make sure to mark if absolute ethanol 60ml is added or not. It should be stored at room temperature. *Caution : Put the lid back on to prevent ethanol vaporization.	
Washing buffer 2 (**W2**)	50 ml
W2 is supplied as a concentrate. At first use, make sure 세 mark if absolute ethanol 200ml is added or not. It should be stored at room temperature. *Caution : Put the lid back on to prevent ethanol vaporization.	
Elution buffer (**E**)	50 ml
10mM Tris (pH 8.5) Store at room temperature.	

Plate and Cover	
96 well binding plates	2ea
96 well dome plates	2ea
96 well RV plates	2ea

III. Required reagents and Equipments

1. Absolute ethanol (98 ~ 100%)
2. Absolute isopropyl alcohol
3. Incubator
5. 96 well vacuum block
6. Multichannel Pipet with Tip
7. Disposable Gloves

IV. Caution before Starting

Before you proceed, check if you have done the below.

1. Make Proteinase K stock solution.
Dissolve Proteinase K in 1.25mL of diluted water.

2. Binding Buffer (B, Store at room temperature)
Mix and use before adding Binding buffer

3. Washing buffer 1(W1, Store at room temperature)

W1 buffer is provided in concentrated liquid form. Add 60mL of alcohol (96~100%) at first use.

4.Washing buffer 2(W2, Room temperature storage)
W2 buffer is provided in concentrated liquid form. Add 200mL of alcohol (96~100%) at first use.

4. Elution buffer (E, Room temperature storage)
Keep the buffer warm at 60 ℃ before an experiment.

5. Controlling of Vacuum block

Measure vacuum after new binding plate is equipped on the tray of vacuum block. Vacuum measure should be between 200 and 350mmHg.
High vacuum results in sputtered solution and low vacuum caused filtering time to be delayed
The following is a method-controlling vacuum. Put tray at the bottom of vacuum block and place a new binding plate on it.
Pour 100uL of diluted water and measure vacuum. It should be dried completely before another use.

V. Experimental Protocol

V-1 Extraction of Genomic DNA from Whole blood (Buffy coat or cultured cells)

1. Put 20uL of Proteinase K to each well of 96 well dome plate.

Mix Proteinase K stock Solution before use.

2. Add samples to 96 well dome plate prepared at step 1. (Start with 10^4~10^8 cell/ml in cultured cell and 200uL in whole blood cell)

If the volume of sample is less than 200 uL, meet 200 uL by adding PBS buffer.

3. After adding 200uL of binding buffer(B) to each well of 96 well dome plate containing sample, cover the plate with provided sealing tape.

Avoid wetting the rims and cross-contamination.

4. Proceed the reaction by shaking for 20mins at 37℃.

Performing reaction with 96 well shaker to shake will help Lysis process.

Shaking at 60℃ may shorten the reaction time.

5. After the reaction is complete, remove sealing tape and put 100ul of Isopropanol to each well and mix them gently for 15sec.

It is more effective to use 96 well shaker.

6. Place the waste tray in the vacuum manifold and binding plate in upper position.

7. Transfer prepared sample to 96 well binding plate.

Avoid cross-contamination during move.

8. Start vacuuming with vacuum pump.

Keep the vacuum with regular pressure and observe the solution if it is passing through well.

9. Stop vacuum pump after solutions in 96 well binding plate is completely removed.

10. Add washing buffer A(W1) to each well of plate.

11. Start vacuuming (3~5min.)

12. Stop vacuum pump after solution in 96 well binding plate is completely removed.

13. **Remove the 96 well binding plate equipped on the top of Vacuum Manifold (BioVac 96), and the waste tray inside the instrument, then discard the filtrate.**
    While removing the 96 well Binding plate installed on the top, the filtrate may remain at the bottom of the plate, therefore, carefully wipe off the droplets using Kimwipes.

14. **While eliminating the droplets using Kimwipes, you must be cautious that the solution does not splash onto other staffs**.
    There is no need to completely remove ethanol during the W1 treatment step.

15. **Install waste tray inside Vacuum Manifold (BioVac96), and then install 96 well Binding plate on the top of it.**

16. **Add 500uL of Washing buffer B (WB) to each well of 96 well Binding plate.**

17. **Perform Vacuum filtering by operating vacuum pump (5 minutes)**

18. **When the solution in 96 well binding plate is completely removed, stop the vacuum pump.**

19. **Remove 96 well binding plate installed on the top of vacuum manifold (*BioVac 96*), and remove waste tray, then discard the filtrate**.
    While removing the 96 well Binding plate installed on the top, the filtrate may remain on the bottom of the plate, therefore, carefully wipe off the droplets using Kimwipes.

20. **Wrap the bottom of 96 well Binding plate Completely**

using 4~5 pieces of Kimwipes, and shake off the plate, and remove the remaining solution.

While eliminating the droplets using Kimwipes, you must make be cautious that the solution does not splash onto other staffs.

You should check out the remaining ethanol at the bottom of the plate.

21. Repeat washing Buffer 2(W2) step one more time.

22. Install 96 Well Binding plate on Vacuum Manifold (BioVac96) again, and completely remove the remaining ethanol by vacuuming for 5~6 minutes

The remaining ethanol may influence the sequential steps after DNA extraction, so it is important to carry out this step throughly.

23. Stop the vacuum pump, and repeat step 20.

Check out whether there are small droplets hanging on each well of the bottom of 96 well binding plate or not, all droplets may be eliminated using Kimwipes.

24. Install 96 well dome plate on the bottom of Vacuum Manifold (BioVac96), and install 96 well Binding plate on the top of it.

Check the code of each well, which is the combination of an alphabet and an Arabic number, and make sure that all samples are in the correct position.

25. Add 200uL of Elution Buffer (E) to 96 well Binding plate, and then store at room temperature for about 1 min.

To obtain high DNA recovery yield, pre-heat the elution buffer(E) at 60 ℃ before use.

Add elution buffer (E) in the middle columns of 96 well

binding plate.

26. Start vacuum filtering by operating vacuum pump (1~2 minutes)

27. Stop the vacuum pump if the solution in 96 well binding plate is completely removed.
Purified genomic DNA can be directly used in researches and experiments, or stored at 4 ℃ for later use, and at -20℃ for longer storage.
About 6ug(30ng/uL) of genomic DNA may be obtained from about 200uL of blood sample.

VI. Problem Solving

1. Low yield of recovery or low DNA preparation

1) Store the kit at 15 ~ 25 ℃

2) Especially, store the solution covered. The quality and stability of buffer may decrease according to pH measure and contamination. In the case of powder reagents, dissolve and divide into smaller parts, then store at -15 ~ -25 ℃.

3) Do not use more ethanol than the recommended amount. In addition, mark the total volume after adding ethanol. In order to prevent ethanol evaporation, shut the cap and store at 15 ~ 25 ℃.

4) All reagents and solution should be mixed completely before use. In addition, add buffer to each tube, then mix sufficiently.

5) Check out the pressure of vacuum pump.
   If the degree of vacuum is too high, the yield of recovery may decrease.

2. Low yield of DNA recovery after elution

When using inappropriate buffer (For instance: distilled water etc.) for elution or incorrect pH values, the yield of recovery may decrease. For more efficient DNA recovery, the elution buffer of the alkaline pH provided in the kit is recommended.

3. Incomplete restriction treatment endonuclease of extracted DNA

1) Since binding Buffer (B) may not be completely eliminated, repeat washing buffer 2(W2) procedure one more time.

2) Ethanol may not be completely eliminated, therefore, perform vacuuming for 1~2 minutes after washing step.

4. Low amount of extracted DNA

1) Completely dissolve proteinase K powder before use

2) Completely lysis the samples, add proteinase K, and then mix with samples. Completely mix by adding isopropanol when adding the mixture to column filter tube.

5. Solution eluted from Blood is slightly colored.

1) Since the cell lysis is not completed, check the binding buffer (B) step.

2) Complete lysis depends on the activity of offered Proteinase K. If possible, avoid repeated freezing and thawing of Proteinase K stock solution.

3) The washing step was not thoroughly done. Wash more time until the color disappears.

6. White precipitants appear in Binding Buffer(B)

If the Binding Buffer(B) was put at lower temperature condition for a longer period, white precipitants will be formed in Binding Buffer(B),
In this case, the precipitants can be dissolved at 60 °C. The precipitants have no effect on the performance capability of the kit.

VII. Supplement

Common yield of Kit

The yield and purity of extracted Genomic DNA may vary according to sample type and condition.

VIII. Reference

1. Boom, R. *et al.* (1990), *J Clin. Microbiol.*, **28**, 495-503

2. Carter, M.J. and Milton, I.D. (1993) *Nucleic Acids Res.*, **21**, 1044

3. Melzak, K. A. *et al*. (1996), *J Colloid and Interface Sci.*, **181**, 635-644

4. Taylor,R. G., Walker, D. C. and Mclnnes, R. R. (1993) *Nucleic Acids Res.*, **21**, 1577-1678

5. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *MolecuLar Cloning* 2nd ed.

BIONEER CORPORATION

Headquarters
49-3,Munpyeong-dong,
Daedeok-gu,Daejeon306-220,
Korea
Phone:+82-42-936-8500
Fax:+82-42-930-8600

Seoul Office
412, 1580, DMC Hi-tech industry
center, Sangam-dong, Mapo-gu,
Seoul, 121-835, Republic of Korea
Phone:+82-2-598-1094
Fax:+82-2-598-1096

USA Bioneer, Inc.
1000 Atlantic Avenue, Alameda, CA
94501 USA
Toll free : 1-877-264-4300
Fax : 1-510-865-0350
E-mail:order.usa@bioneer.com

Order
Domestic only: 1588-9788
E-mail: order@bioneer.co.kr
URL: www.bioneer.com